Iranian Journal of Veterinary Science and Technology

Received: 2020- Nov- 05
Accepted after revision: 2020- Nov- 25
Published online: 2021- Feb- 27

RESEARCH ARTICLE

DOI: 10.22067/ijvst.2020.39239

# Effects of single injection of vitamin D3 on some immune and oxidative stress characteristics in transition dairy cows

Morteza Hassanabadi[a], Mehrdad Mohri[a,b], Hesam A Seifi[a,b]

[a] Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
[b] Center of Excellence in Ruminant Abortion and Neonatal Mortality, Ferdowsi University of Mashhad, Mashhad, Iran.

## ABSTRACT

Recent studies suggest that vitamin D may have preventive and therapeutic effects on autoimmune disease, cancer, and diabetes type 1 and 2 beyond the skeletal condition and calcium metabolism. To demonstrate the effects of an over-supplemented single 8 million I.U. vitamin D3 IM injection on the modulation of immune responses and oxidative/antioxidative variables in transition dairy cows, this study was conducted on a commercial dairy farm with about 1500 lactating cows in the Tehran province, Iran. Twenty-four multiparous Holstein cows were randomly categorized into control and treatment groups. In the treatment group, 12 cows received a single dose of 8,000,000 IU vitamin D intramuscularly. In the control group, a placebo (distilled water) was injected into 12 cows 2 to 8 days before the expected calving time. Blood samples were collected on 21 and 7 days before calving and 1,3,7,15, and 30 days after calving. 25(OH)vitamin D3, tumor necrosis factor- α (TNF-α), interferon-γ (INF-γ), haptoglobin, interleukin 6 (IL-6), ferric reducing the ability of plasma (FRAP), glutathione peroxidase (GPx), superoxide dismutase (SOD), and hemolysate GPx were measured. This study showed that the treatment group had significantly higher amounts of 25(OH) vitamin D3, hemolysate GPx, and IL-6 values than the control group. According to our results, vitamin D3 injection increased the amounts of IL-6 and hemolysate GPx activity and tended to affect serum GPx activity.

**Keywords**

*Haptoglobin, immune regulation, superoxide dismutase, 25(OH) Vitamin D*

## Abbreviations

DMI: Dry matter intake
Mcal: Mega calorie
DCAD: Dietary anion cation difference

Number of Figures : 2
Number of Tables : 4
Number of References : 35
Pages : 25-35



*Corresponding author:* *Mehrdad Mohri*
*Email:* *mohri@um.ac.ir*
*Tel:* *+98 (51)3880 -5643*

## Introduction

For many years, vitamin D has been known for its classical effects on calcium (Ca) metabolism and and skeletal turnover. Initial evidence for non-classical effects of the active form of vitamin D (1, 25-dihydroxy vitamin D) arose from studies in the early 1980s. Recent studies in humans suggest that beyond the skeletal effects and Ca metabolism, vitamin D may have preventive and therapeutic effects in cardiovascular and autoimmune diseases, common cancers, and type 1 and 2 diabetes [1, 2].

Vitamin D classical target organs include bones, intestine, kidney, and parathyroid glands. Vitamin D promotes active uptake of calcium to regulate calcium concentrations within normal limits. The epithelial calcium channel, transient receptor potential vanilloid 6 (TRPV6), and calbindin transport of calcium into the cells are upregulated by vitamin D [3]. Most studies in cattle are toward regulating calcium homeostasis by vitamin D linked to the onset of lactation and hypocalcemia after parturition (milk fever) [4, 5]. After parturition, most cows undergo negative calcium balance. Thus, transition period management is an important step to prevent many diseases in dairy cows, and maintaining calcium in a normal range is necessary [6].

Vitamin D receptor (VDR) is widely distributed in many organs such as the heart, stomach, pancreas, brain, skin, and glands. Activated T and B lymphocytes and macrophages also have nuclear receptors for Vitamin D. Many autoimmune diseases including type 1 diabetes, rheumatoid arthritis, and multiple sclerosis have all been successfully prevented by receiving 1,25 $[OH]_2D3$ early in life [7].

Vitamin D signaling in immune cells can improve innate and adaptive immune reactions. Vitamin D influences the adaptive immune system in cattle similar to that in humans, but the target of vitamin D in the innate immune system is somewhat different. Recent studies in cattle and humans showed that vitamin D inhibits the pro-inflammatory effect of interferon-γ (INF-γ) and interleukin -17 (IL- 17) secreted by antigen-specific T cells *in vivo* [8, 9]. Vitamin D also plays a role in cattle innate immune system mediated by Toll-like receptors that upregulate vitamin D receptors and CYP27B1 (1α[OH]ase), the enzyme that converts 25[OH]vitamin D3 to 1,25$[OH]_2D3$. Vitamin D induces the expression of VDR on macrophages and phagocytes. In cattle and humans, vitamin D is responsible for the upregulation of nitric oxide (NO) gene expression and increasing NO production [2, 8].

1, 25 $[OH]_2D3$ affects the activity of antigen-presenting cells (APCs), which act as a mediator between innate and adaptive immunity. 1,25$[OH]_2D3$ induces 'tolerogenic' dendritic cells, [DC] down-regulates CD40 (required for B cell activation), elevates IL-10 production in DC, and down-regulates MHC class II molecule expression on APCs [9].

Studies have shown that a high dose of vitamin D can prevent type 1 diabetes by immune regulation. Vitamin D is a potent blocker of dendritic cell differentiation that directly blocks IL-12 secretion [1, 10]. Vitamin D deficiency has long been linked to glucose intolerance in humans. Many studies in humans have shown that vitamin D plays an important role in the pathogenesis of type 2 diabetes by affecting either insulin sensitivity or β cell function, or both [10, 11].

The dose of vitamin D supplementation effective in dairy cow health is not well defined. The NRC (2001) recommends 21,000 IU supplemental vitamin D3/day (~800 to 1,000 IU/kg of DM) for lactating Holstein cows (calculated for 680 kg of BW). Instead, dairy producers typically provide lactating cows with 30,000 to 50,000 IU of vitamin D3 [12]. Nelson et al. clarified that 22% of cows supplemented with 20,000 IU/day vitamin D (NRC recommendation) had serum 25[OH] D below 30 ng/mL whereas, 95% of cows receiving 30,000 IU/d or more have serum 25[OH] D above 40 ng/mL [12]. Nelson et al. showed that most dairy cows that received vitamin D 1.5 to 2.5 times more than the recommended NRC vitamin D have an average serum vitamin D of 60 to 70 and in the range of 40 to 100 ng/ml [12]. Lean et al. suggested 40,000 IU vitamin D for appropriate vitamin D functional role but considering optimal time supplementation before calving is a vital step [13]. A recent study has shown that serum vitamin D concentration is diminished in the transition period, particularly in early lactation when dairy cows are most susceptible to disease and metabolic stress. Diminished vitamin D concentrations may enhance inflammatory reaction and oxidative stress [14]. Early studies have shown that NRC recommendation for vitamin D supplementation may not be enough for a proper immune function. Moreover, in dairy cows vitamin D concentrations have been reduced in critical periods just after calving [12, 14]. In early lactation, cows are vulnerable to metabolic diseases and oxidative stress, so the reduced serum vitamin D may play a role in this condition. The effects of vitamin D on immune system regulation and energy balance are well known in human medicine, but there is still a lack of knowledge in veterinary research in this field [9].

Dysregulated inflammation and adipose remodeling are recognized as the key components of the metabolic stress syndrome and cytokines secreted in the lipolysis process, particularly after parturition [15]. The present study aimed to demonstrate the effects of an over-supplemented single 8 million I.U. vitamin D3 IM injection is variable on the modulation of immune responses and oxidative/antioxidative status during the transition period of dairy cows.





## Results

This study showed that injection of vitamin D significantly increased 25(OH) vitamin D, IL-6, and hemolysate GPx values in the treatment group (Figurs 1 and 2, Table 1, $p \leq 0.05$) in comparison with control cows. Time of sampling (time) did not have a significant effect on any of the measured variables. There was not any treatment × time interaction for measured variables. BCS and number of parturition did not have significant effects as the covariate.

Pairwise comparisons for each sampling time showed that serum GPx at 7 days after calving, hemolysate GPx at 7 and 30 days after calving, and SOD at 15 days after calving caused a significant difference between trial groups (Figurs 1 and 2, Table 1, $p \leq 0.05$).

## Discussion

Serum vitamin D concentration is reduced in early lactation due to increased demand in calcium homeostasis and consumption by the immune system. This condition may be associated with metabolic diseases after calving [14]. In the present study, the injection of vitamin D prevented the reduction of vitamin D in early lactation in the treatment group, compared to that in the control group.

Many studies proved that vitamin D enhances innate immunity by stimulating antimicrobial peptides, such as β-defensin, nitric oxide, cathelicidin, pattern recognition receptors, and cytokines [16,17]. Vitamin D may downregulate TLR-2 and TLR-4 expression and cause hyporesponsiveness to the pathogen-associated molecular pattern molecules (PAMPS), and the controlled immune responses [2]. In the present study, total globulin in the treatment group did

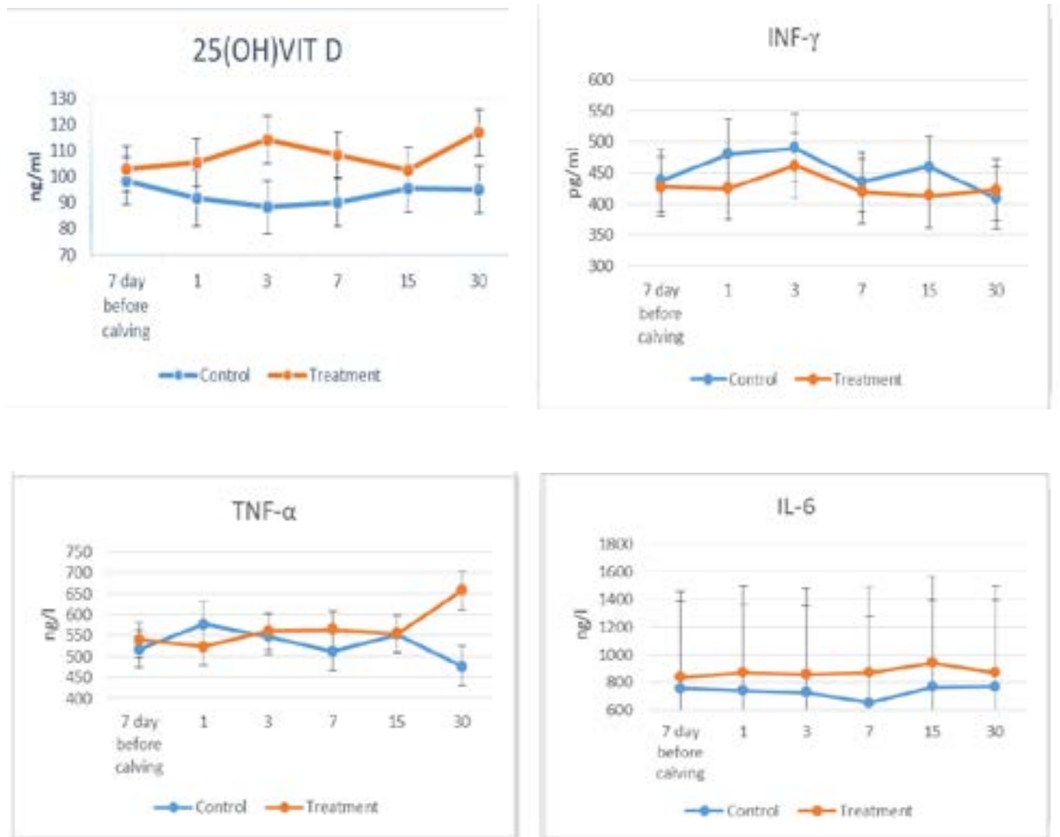


**Figure 1.**
Time related changes (LSM ± SE) and pairwise comparisons for the amounts of 25(OH) vit D, TNF- α, INF-γ, and IL-6 in trial groups.

**Table 1.**
Least square mean and SE of measured variables and the effects of time and treatment in trial groups

| Variables | Control (n=12) | Treatment (n=12) | SE | Treatment effect | Time effect | Time*Treatment effect |
|---|---|---|---|---|---|---|
| 25(OH)Vitamin D (ng/ml) | 93.21 | 108.41 | 3.94 | 0.01 | NS | NS |
| Total-globulin (g/dl) | 3.81 | 3.99 | 0.08 | NS | NS | NS |
| INF-γ (pg/ml) | 452.10 | 428.39 | 20.9 | NS | NS | NS |
| TNF-α (ng/l) | 530.21 | 566.71 | 21.07 | NS | NS | NS |
| IL-6 (ng/l) | 736.07 | 875.62 | 618.07 | 0.04 | NS | NS |
| Haptoglobin (µg/ml) | 203.14 | 208.28 | 5.21 | NS | NS | NS |
| GPx (U/ml) | 948.07 | 1010.91 | 22.78 | 0.06 | NS | NS |
| hemolysate GPx (U/mg Hb) | 1385.79 | 1639.59 | 189.49 | 0.003 | NS | NS |
| SOD (IU/ml) | 293.15 | 295.67 | 4.46 | NS | NS | NS |
| FRAP (mmol $Fe^{2+}$/L ) | 16.13 | 16.33 | 0.36 | NS | NS | NS |

S: significant difference ($p \leq 0.05$), NS: not significant

not show any significant difference with the control group. According to the results concerning the globulin amount, a previous study suggested that vitamin D has a greater tendency to promote cell-mediated T helper1 immunity than antibody-mediated T helper 2 immunity [2].

TNF-α, IL-6, INF-γ are the major cytokines in immune regulation. Borges et al. proposed that the immune-modulatory effects of vitamin D are associated with a reduction in the INF-γ level [18]. Adam and Hewison (2008) suggested that vitamin D could suppress T helper proliferation and also inhibit Th1 cytokine expression (INF- γ, TNF- α, IL-2) [2]. In this regard, our results for INF-γ are similar to the previous studies. The treatment group had lower INF-γ mean value than the control group, although it was not significant (control group =452.1, treatment group= 428.39). The results of the present study were in agreement with the previous studies showing that vitamin D supplementation could not significantly diminish INF-γ value [19, 20]. There are some possible explanations for non-significant INF-γ changes in the present study. Firstly, in the absence of active inflammation, the immunomodulatory effect of vitamin D on the levels of INF- γ did not occur [19]. Secondly, the small number of animals in each group may have reduced the statistical power for comparison to find a significant difference between the two groups. On the other hand, in the present study, TNF-α amount was numerically higher in the treatment group. In addition, the IL-6 levels were significantly higher in the treatment group. IL-6 and TNF-α are involved in innate immunity and are produced by T helper1 cells and macrophages [18]. The previous studies demonstrated that TNF-α and IL-6 were important cytokines produced in visceral adipose tissues [18,21,22]. The transition period in dairy cows is a critical time for the occurrence of inflammation and many inflammatory conditions experienced during this period, including metabolic and infectious diseases, stressful situations, trauma at calving time, energy excess or deficit, and digestive upsets [23]. In dairy cows, adipose tissue plays an endocrine role. There is a hypothesis that cows with higher (obese) body scores develop inflammation, which leads to insulin resistance and impaired insulin secretion [24]. Our results about IL-6 are different from the previous studies in humans. An earlier study revealed that vitamin D could diminish IL-6 and TNF-α concentrations [18]. Incubation of isolated monocytes with $1,25[OH]_2D3$ attenuates the expression of pro-inflammatory cytokines involved in insulin resistance such as IL-1, IL-6, and TNF-α in type 2 diabetes mellitus human patients [25]. Also, in dairy cows and other ruminants, macrophages of adipose tissue and lipolysis mechanism (adipose tissue remodeling) cause the activation of NF-κB signal transduction and induce IL-6, TNF-α, and IL-1 secretion, especially during the dairy cow transitional period [15]. Although IL-6 was significantly increased in the treatment group, there were no significant differences in the occurrence of inflammatory diseases during the study between the groups.





**Table 2.**
Ingredients, chemical composition and nutritive value of diets fed to close up cows

| | DMI | % | | |
|---|---|---|---|---|
| Legume hay immature | 4.17 | 32.61 | Nel 3X (Mcal) | 19.45 |
| Corn silage immature | 2.42 | 18.97 | Nel 3X (Mcal/kg) | 1.53 |
| Wheat straw | 0.46 | 3.63 | RUP Kg | 0.74 |
| Corn gluten meal | 0.13 | 1.01 | RUP % of DM | 5.84 |
| Extrude linseed (flax) | 0.41 | 3.21 | RDP kg | 0.74 |
| Barley grain rolled | 1.35 | 10.57 | RDP % of DMI | 1.12 |
| Corn grain ground dry | 1.50 | 11.71 | CP Kg | 1.86 |
| Wheat bran | 0.13 | 1.05 | CP % DM | 14.6 |
| Canola meal | 0.45 | 3.53 | NDF % | 37.31 |
| Cotton seed meal (solvent extracted) | 0.32 | 2.49 | ADF % | 23.01 |
| Soybean meal, expellers | 0.36 | 2.79 | NFC % | 39.81 |
| Full-fat soy, roated | 0.19 | 1.46 | EE % | 4.73 |
| Fish meal, anchovy | 0.18 | 1.44 | Ca/P | 3.48 |
| Vegetable oil | 0.15 | 1.17 | Absorbable Ca (Kg/day) | 0.16 |
| Anionic supplement * | 0.50 | 3.91 | Dietary Ca % | 1.24 |
| Limestone | 0.02 | 0.15 | Absorbable P (Kg/day) | 0.05 |
| Toxin blinder | 0.02 | 0.12 | Dietary P % | 0.36 |
| Availa chrome | 0.01 | 0.06 | Mg % | 0.57 |
| Levucell | 0.02 | 0.12 | Cl% | 0.87 |
| Total | 12.78 | 100.00 | K% | 1.44 |
| Forage % of DMI | 55.33 | | Na % | 0.04 |
| Concentrate % of DMI | 43.67 | | S% | 0.35 |
| | | | DCAD (mEq/Kg Dm) | -81.46 |

*refer to Table 4

An *in vitro* study suggested that the effects of vitamin D on IL-6 expression in monocyte/macrophage could be slightly upregulated, depending on various factors, such as interleukin under consideration, the degree of maturation, and the stimulus. This study showed that vitamin D in monocyte/macrophage in the absence of TNF-α could markedly induce IL-6 gene expression [26]. Naghavi Gargari et al. showed that vitamin D supplementation up-regulated IL-6 and IL-17A gene expression in multiple sclerosis patients [27]. Vitamin D could affect IL-6 expression in multiple routes, including NFκB inhibition, p38 MAP kinase inhibition, and CoX-2 inhibition [28]. Both *in vivo* and *in vitro* studies showed that oxidative stress might cause underlying diseases in the transition period of dairy cattle [29, 30]. Oxidative stress causes a dysfunctional inflammatory reaction, raises IL-6 and TNF-α, and induces metabolic stress, which is important for the occurrence of underlying diseases, such as ketosis and fatty liver [31]. GPx represents a main intracellular anti-oxidant agent. The treatment effect tends to be significant for GPx value ($p < 0.06$) in the present study. A previous study in cattle showed that raising intracellular GPx indicated a proper condition against oxidative stress [30]. In addition, in the present study, hemolysate GPx was significantly higher in the treatment group. Thus, vitamin D administration could lead to lower inflammatory and oxidative stress due to better anti-oxidative performance. In our study IL-6, GPx in hemolysate, and probably GPx in serum amounts were significantly higher in vitamin D injected cows, although INF- γ, and TNF-α

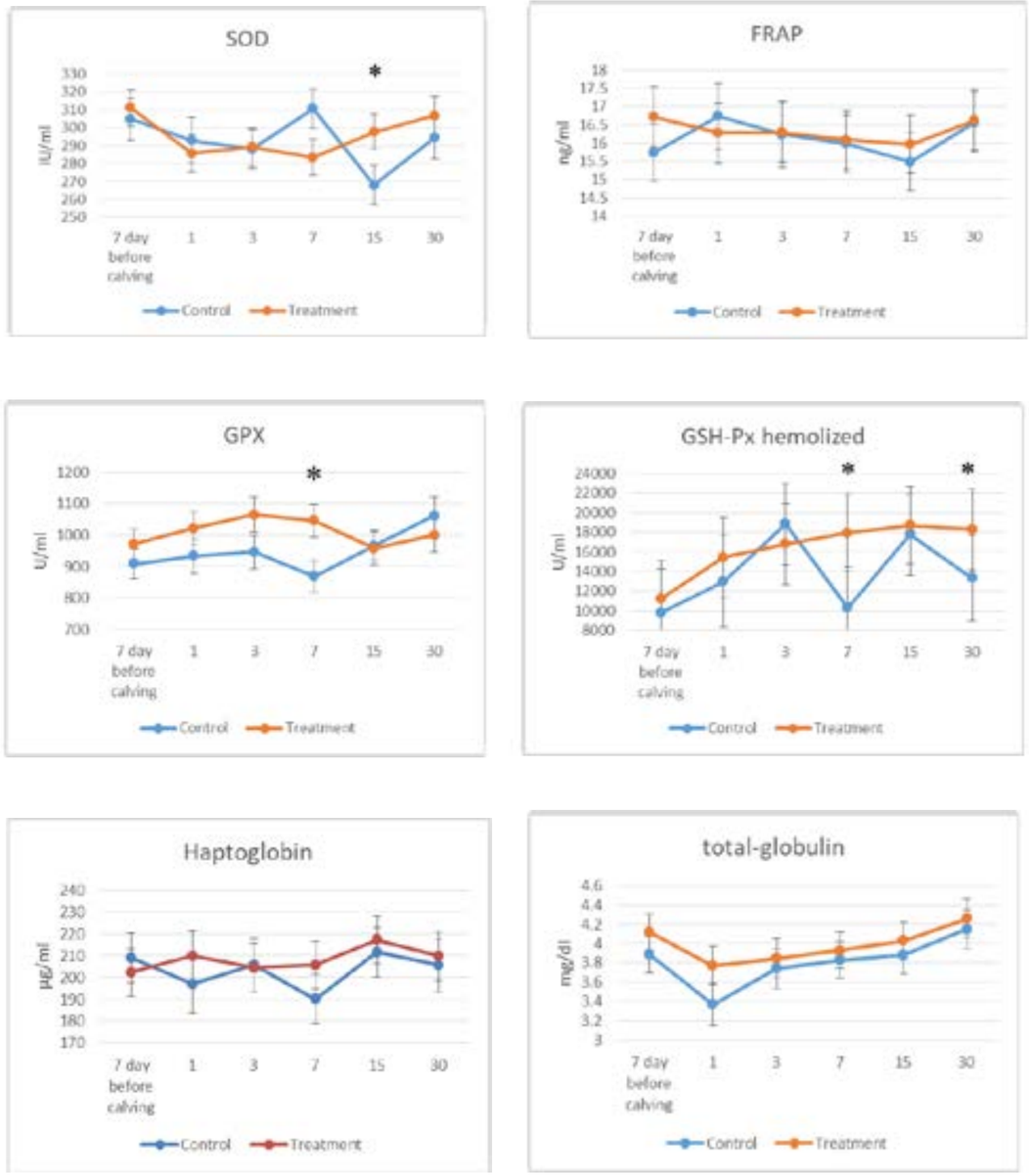


**Figure 2.**
Time related changes (LSM ± SE) and pairwise comparisons for the amounts of GPx, hemolysate GPX, FRAP, SOD, total globulin, and haptoglobin for trial groups. The asterisk shows significant difference between groups ($p \leq 0.05$).





amounts were not significantly different between trial groups. It seems that the increased amounts of GPx activity in serum and hemolysate similar to an increased amount of IL-6 were compensatory responses; however, its precise reason was not clear.

In conclusion, vitamin D injection increased the amounts of IL-6 and hemolysate GPx activity and tended to affect serum GPx activity. The number of animals used in this study was not large enough to show the exact differences between trial groups. Thus, it is necessary to conduct further studies with a larger number of animals to clearly understand the role of vitamin D as an immunomodulatory factor in transition dairy cows.

## Materials & Methods

### *Cows, experimental design, and feed*

The trial was conducted in a commercial dairy farm with about 1500 lactating Holstein cows in Tehran province, Iran. The rolling herd average for milk production was about 10,920 Kg. The herd used mix loose pens with adjacent outside yards and free-stall facilities with sand bedding. The animals had free access to water throughout the experiment. The predominant forages used in this farm were alfalfa hay, and corn silage, and the main concentrates consisted of corn, barley, soybean meal, canola meal, linseed meal, cottonseed meal, wheat bran, gluten feed, and sugarcane bagasse. Feed composition details for both close-up and early lactation cows are depicted in Tables 2 and 3. Total mixed ration (TMR) was offered to cattle twice a day and mixed by a mechanized feeder. Diet's ingredients included between 20,000 and 30,000 IU of Vitamin D3 per day. The farm held a policy of drying off the cattle 8 weeks before the expected calving day. All the cows in close up period were placed on a diet with anionic salts according to table-3 (500g/head/day) including ammonium sulphate, magnesium sulphate, and calcium chloride and a DCAD between -100 to -110 mEq/Kg of DM (Calculated as $(Na^{+} \times 435mEq + K^{+} \times 256mEq) - (Cl^{-} \times 282mEq + S^{2-} \times 624mEq)$). Cattle urine pH were measured twice weekly using a digital pH-meter (Jenway, Model 3040, England) from 2 days after the anionic salts were added until calving to make sure that the value was never less than 5.8 or more than 6.8.

Twenty-four Holstein cows were randomly selected and put in control and treatment groups. Blood sampling was conducted in the same season and time in the treatment and control groups. Both groups were approximately homogenous concerning body condition score (BCS) and number of parturition of cows. In the treatment group, 12 cows received a single dose of 8,000,000 IU vitamin D3 (cholecalciferol, Darou Pakhsh Co., Iran) intra muscularly before sampling at seven days before the expected calving time, and in the control group, 12 cows were injected placebo (injectable distilled water) at the same time. In our study, the dose of vitamin D was selected based on previously published studies and also for preventing of any probable toxicosis [32, 33]. Cows that did not calve within the expected time were eliminated from the study; the elimination criterion also included those who lost their health for any reason during the study. The parity of the cows ranged from 3 to 7 and its average for control and treatment groups was 4.42 and 3.83, respectively. The body conditions of all cows were scored in far-off period based on a 5-point scale and an increment of 0.25 (34). All scorings were performed by a single evaluator. All the cows in both groups were clinically healthy based on Duffield et al. and had a BCS between 3.25 and 4 initially in the far-off period [35]. The cows were also categorized into 2 groups based on BCS; BCS of ≥ 3.7 (control, n=5, and treatment, n=4) were enrolled as fat cow (FC), and a BCS of < 3.7 (control, n=7, and treatment, n=8) as nonfat cow (NFC) for statistical purposes.

### *Blood sampling and variables measurements*

Blood samples were collected via jugular vein with disposable syringes between 8 to 10 A.M on 21 and 7 days before calving and 1,3,7,15, and 30 days after calving. We considered 21 days before calving as covariate for other sampling times. Blood samples were taken by disposable syringes on the plain tube and chilled immediately after collection, and sera were harvested immediately after centrifugation at 2200×g for 10 min. Serum was kept frozen at −20°C until transfer to the laboratory for further analysis. EDTA containing tube was also used for obtaining whole blood. Hemolysate was prepared as follows: After primary centrifugation of whole blood (1800 g for 15 min) plasma was removed and the remaining packed erythrocytes were washed three times with normal saline. Eventually four volumes of cold distilled water were added to one volume of the washed packed erythrocytes, shaken and after final centrifugation; supernatant hemolysate was aliquoted and stored at -20°C. 25(OH)Vitamin D, tumor necrosis factor-α (TNF-α), interferon-γ ( INF-γ), haptoglobin, interleukin-6 (IL-6), and glutathione peroxidase (GPx) were measured in serum by cattle-specific enzyme-linked immunosorbent assay kits (Shanghai Crystal Day Biotech CO., LTD, Shanghai, China). Superoxide dismutase (SOD) and ferreic reducing agent of plasma (FRAP) in serum and GPx in hemolysate were also measured by commercial kits based on enzymatic reactions (Randox Laboratories Ltd., Ardmore,UK). Intra-assay and inter-assay coefficient of variation for all ELISA tests were less than 8% and 10%, respectively, and sensitivities are as following: 25(OH)Vitamin D: 0.53 ng/ml, TNF-α: 5.56ng/L, IL-6: 10.56 ng/L, INF-γ: 2.35 pg/ml, haptoglobin: 1.36 µg/ml, and GPx: 5.69 U/ml. The inter assay and intra assay of SOD measurement method were 7.07% and 3.58%, respectively, and the sensitivity was < 6.13 U/ml. The total assay and intra assay of GPx measurement method were 4.37% and 3.2%, respectively, and the sensitivity was 75 U/L. FRAP kit sensitivity was 2 µmol $Fe^{2+}$. All measurements were performed by biochemical auto-analyzer (Biotecnica, BT 1500, Rome, Italy). Control serum (Randox Laboratories Ltd., Ardmore, UK) was used for controlling measurement accuracy. For measuring of globulin amount, serum total protein, and albumin concentrations were measured by commercial kits (Pars azmoon, Tehran, Iran) and the amount of globulin was calculated by subtracting of albumin from total protein.

### *Data management and statistical analysis*

Normality of variables was evaluated by PROC UNIVARIATE of SAS software, version 9.2 (SAS Inst. Inc., Cary, NC). Variables with Shapiro-Wilk values $p > 0.05$ were considered normal (25(OH)Vitamin D, TNF-α, INF-γ, IL-6, GPx, hemolysate GPx, SOD, total globulin) while all other variables were normalized using natural logarithm.

Data of serum profile and cytokines were then analyzed using repeated-measures ANOVA (Mixed procedure in SAS, version 9.2). The model for all serum metabolite contained the effects of time of sampling (-7, 1, 3,7,15, 30 days), BCS category, and parity group. Cows that had a BCS of ≥ 3.7 were enrolled as fat cow (FC), and a BCS of < 3.7 as nonfat cow (NFC). Parity was classified into 2 groups: cows with third and fourth parity as parity group 1 (control, n = 8, and treatment, n = 10) and cows with five and higher parity as parity group 2 (control, n = 4, and treatment, n= 2). Interactions between time of sampling and BCS were tested to see if BCS effect was significant. Pairwise comparisons for each

sampling time were conducted by *Tukey's* procedure. Differences with $p \leq 0.05$ were considered as significant, and $0.05 < p \leq 0.10$ were considered as a tendency. Least square means and standard errors are presented as LSM ± SE.

### *Animal Welfare Statement*

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee of Ferdowsi University of Mashhad approval has been received (3/43622). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

**Table 3.**
Ingredients, chemical composition and nutritive value of diets fed to fresh cows

| | DMI | % | | |
|---|---|---|---|---|
| Alfalfa hay | 3.704 | 17.02 | NEL 3X (Mcal) | 36.09 |
| corn silage | 2.83 | 12.99 | NEL 3X (Mcal/kg DM) | 1.85 |
| Wheat straw | 0.185 | 0.85 | RUP (kg) | 1.75 |
| Corn gluten meal | 0.518 | 2.38 | RUP % of DMI | 8.06 |
| extruded linseed (full-fat flaxseed)1 | 0.446 | 2.05 | RDP (kg) | 1.83 |
| Barley grain rolled | 2.71 | 12.41 | RDP % of DMI | 8.39 |
| Corn grain ground dry | 2.64 | 12.13 | CP kg | 3.58 |
| Wheat bran | 0.21 | 0.94 | CP % of DM | 16.45 |
| Canola meal | 0.155 | 5.31 | NDF % of DMI | 33.73 |
| Cotton seed W lint | 0.531 | 2.44 | ADF % of DMI | 19.33 |
| Soy Meal, expellers, | 1.682 | 7.73 | NFC % of DM | 43.05 |
| Soybean seeds, whole roasted | 0.344 | 1.58 | EE % of DM | 4.68 |
| Fish meal, Anchovy | 0.165 | 0.76 | Absorbable Ca (Kg/day) | 0.18 |
| vegetable oil | 0.21 | 0.96 | Dietary Ca % of DMI | 0.82 |
| beet Pulp, dried | 3.532 | 16.23 | absorbable P (Kg/day) | 0.11 |
| Calcium Carbonate | 0.089 | 0.41 | Dietary P % of DMI | 0.51 |
| DCP (Di-Calcium Hydrogen Phosphate) | 0.048 | 0.22 | Ca/P ratio | 1.62 |
| Sodium Bicarbonate | 0.181 | 0.83 | Mg % DMI | 0.34 |
| Salt | 0.039 | 0.18 | Cl % of DMI | 0.36 |
| Magnesium oxide | 0.051 | 0.23 | K % of DMI | 1.64 |
| Vitamin/Mineral supplement6 | 0.161 | 0.74 | Na % of DMI | 0.42 |
| Bentonite | 0.061 | 0.28 | S % of DMI | 0.25 |
| Propylene glycol2 | 0.250 | 1.15 | DCAD (mEq/kg DM) | +345.3688 |
| Antimycotoxin3 | 0.015 | 0.07 | | |
| Yeast4 | 0.015 | 0.07 | | |
| Rumen protected methionine5 | 0.009 | 0.04 | | |
| total | 20.78 | | | |
| Forage% of DM | 30.87 | 100 | | |
| Concentrate % of DM | 66.75 | | | |

1Shayflax, Tehran, Iran
2Glycoline™, Vitalac Co, France
3mycosorb™, Alltech co, USA
4levucell™, (Saccharomyces cerevisiae CNCM I-1077), Nutritech co, USA
5mepron™, Evonik industries, Wien, Austria
6 vitamin/mineral supplement contain: Ca 13.4%, P 1.08%, Mg 3.4%, Na 10.35%, Cl 8.11%, Co 20 mg/kg, Cu 1500 mg/kg, I 70 mg/kg, Fe 3000mg/kg, Mn 4000 mg/kg, Se 370 mg/kg, Zn 5000 mg/kg, Vit A 700000 IU/kg, Vit D 200000 IU/kg, Vit E 2000 IU/kg





**Table 4.**
Ingredients of Anionic salt used in diet of cows in close-up period (ANIOMIX®)

| Ingredient | Amount |
|---|---|
| Vitamin A | 300000 IU |
| Vitamin D3 | 45000 IU |
| Vitamin E | 3000 IU |
| Calcium (Ca) | 150 gr |
| Chlorine (CL) | 150 gr |
| Magnesium (Mg) | 25 gr |
| Sulfur (S) | 35 gr |
| Zinc (Zn) | 1500 mg |
| Manganese (Mn) | 1200 mg |
| Copper (CU) | 500 mg |
| Selenium (Se) | 8 mg |
| Cobalt (Co) | 9 mg |
| Iodine (I) | 12 mg |
| Chrome (Cr) | 14 mg |
| Monensin | 400 mg |
| Niacin (B3) | 4 gr |
| Antioxidant | 1000 gr |
| DCAD | -6473.21 mEq/kg |
| DCAB | -4285.714 mEq/kg |

## Acknowledgements

The authors are grateful for financial support from Ferdowsi University of Mashhad, Deputy of Research and Technology (grant number: 43622). The authors thank Dr. Hamideh Zeinali, Dr. Saba Ahmadi, and Mr. Hossein Barati for assisting in laboratory measurements. We wish to acknowledge the owners and personnel of the collaborating Dehiran dairy farm for allowing us access to the facility and to conduct this research.

## Authors' Contributions

MH contributed to the main design of study, sample collection, laboratory tests, data analysis, and drafting the manuscript. MM contributed to main design of the study, data analysis, and reviewed and edited the manuscript. HAS contributed to the main design of the study and data analysis. All authors approved the final version of the manuscript for publication.

## Conflict of interest

The authors declare they have no conflict of interest.

## References

1. Mathieu RC, Gysemans C, Giulietti A, Bouillon, R. Vitamin D and diabetes. Diabetologia. 2005; 48:1247–1257.
2. Adams JS , Hewison M. Unexpected actions of vitamin D : new perspectives on the regulation of innate and adaptive immunity. Nat. Clin. Pract. Endoc 2008; 4(2):80–90.
3. Hoenderop JG, Nilius B, Bindels RJ. Calcium Absorption Across Epithelia. Physiol Rev. 2005; 85(1):373–422.
4. Goff JP, Horst RL, Littledik ET, Beitz DC. Use 24-F-1,25-Dihydroxyvitamin D3 to prevent parturient paresis in dairy cows. Int. J. Dairy Sci 1988; 71(5):1211–1219.
5. Horst RL, Goff JP, Reinhardt TA. Calcium and vitamin D metabolism in the dairy cow. Int. J. Dairy Sci 1994; 77(7):1936–1951.
6. De Garis PJ, Lean IJ. Milk fever in dairy cows: A review of pathophysiology and control principles. Vet. J 2008; 176(1): 58–69.
7. Holick MF. Vitamin D : importance in the prevention of cancers, type 1 diabetes, heart disease and osteoprosis. Am. J. Clin. Nutr 2004; 79(3):362-71.
8. Nelson CD, Reinhardt TA, Lippolis JD, Sacco RE, Nonnecke BJ. Vitamin D signaling in the bovine immune system: a model for understanding human vitamin D requirements. Nutrients. 2012; 4(3): 181–196.
9. O'Brien MA, Jackson MW. Vitamin D and the immune system: Beyond rickets. Vet. J. 2012; 194(1): 27–33.
10. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J. Clin. Endocrinol. Metab. 2007; 92(6): 2017–2029
11. Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. Am. J. Clin. Nutr 2004; 25(4): 820–825.
12. Nelson CD, Lippolis JD, Reinhardt TA, Sacco RE, Powell JL, Drewnoski ME, et.al. Vitamin D status of dairy cattle: outcomes of current practices in the dairy industry. Int. J. Dairy Sci 2016; 99(12): 10150–10160.
13. Lean IJ, Saun RV, Degaris PJ. Mineral an antioxidant management of transition dairy cows. Vet. Clin. North. Am. Food. Anim. Pract. 2013; 29: 367–386.
14. Holcombe SJ, Wisnieski L, Gandy J, Norby B, Sordillo LM.

Reduced serum vitamin D concentrations in healthy early-lactation dairy cattle. Int. J. Dairy Sci 2018; 3: 1–7.

15. Contreras GA, Clarissa Strieder-Barboza C, Raphael W. Adipose tissue lipolysis and remodeling during the transition period of dairy cows. J. Anim. Sci. Biotechnol 2017; 8: 41-53.

16. Querfeld U. Vitamin D and inflammation. Pediatr. Nephrol., 2013; 28: 605–610.

17. Dimitrov V, White JH. Molecular and Cellular Endocrinology Vitamin D signaling in intestinal innate immunity and homeostasis. Mol. Cell. Endocrinol. 2017; 453: 68–78.

18. Borges MC, Martinin LA, Rogero MM. Current perspectives on vitamin D, immune system, and chronic diseases. Nutr. J., 2011; 27(4): 399–404.

19. Das M, Tomar N, SreenivasV, Gupta1 N, Goswami1 R. Effect of vitamin D supplementation on cathelicidin, INF-γ, IL-4 and Th1/Th2 transcription factors in young healthy female. Eur. j. Clin. Nutr. 2014; 68: 338-343.

20. Jorde R, Sneve M, Torjesen PA, Figenschau Y, Goransson LG, Omdal R. No effect of supplementation with cholecalciferol on cytokines and markers of inflammation in overweight and obese subjects. Cytokine, 2010; 50: 175-180.

21. Ohtsuka H, Koiwa M, Hatsugaya A, Kudo K, Hoshi F, Itoh N, et.al. Relationship between serum TNF activity and insulin resistance in dairy cows affected with naturally occurring fatty liver. J. Vet. Med. Sci. 2001; 63(9): 1021–1025.

22. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends. Immunol. 2004; 25(1): 4–7.

23. Bertoni G; Minuti A, Trevisi, E. Immune system, inflammation and nutrition in dairy cattle. Anim. Prod. Sci. 2015; 55(7): 943–948.

24. De Koster JD, Opsomer G. Insulin Resistance in Dairy Cows. Vet. Clin. North. Am. Food. Anim. Pract. 2013; 29(2): 299–322.

25. Giulietti A, van Etten E, Overbergh L. Monocytes from type 2 diabetic patients have a pro-inflammatory profile.1,25-Dihydroxyvitamin D3 works as anti-inflammatory. Diabetes. Res. Clin. Pract. 2007; 77:47–57.

26. Di Rosa M, Malaguarnera G, De Gregorio C, Palumbo M, Nunnari G, Malaguarnera L. Immuno-modulatory effect of vitamin D3 in human monocyte and macrophage. Cell. Immunol., 2012; 280: 36-43.

27. Naghavi Gargari B, Behmanesh M, Shirvani Farsani Z, Pahlevan Kakhki M, Azimi AR. Vitamin D supplementation up-regulates IL-6 and IL-17A gene expression in multiple sclerosis patients. Int. Immunopharmacol., 2015; 28: 414-419

28. Wöbke TK, Sorg BL, Steinhilber D. Vitamin D in inflammatory diseases. Front Physiol., 2014; 5: 244.

29. Pires JA, Pescara JB, Grummer RR. Reduction of plasma NEFA concentration by nicotinic acid enhances the response to insulin in feed-restricted holstein cows. Int. J. Dairy Sci. 2007; 90(10): 4635–4642.

30. Sordillo LM, Aitken SL. Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. Immunopathol 2009; 128: 104–109.

31. Benedito JL, Abuelo A, Hernández J, Castillo C. The importance of the oxidative status of dairy cattle in the periparturient period : revisiting antioxidant supplementation. J. Anim. Physiol. An. N 2015; 99(6): 1003–1016.

32. Littledike ET, Horst RL.Vitamin D3 toxicity in dairy cows. Int. J. Dairy Sci. 1982; 65: 749-759.

33. Julien WE, Conard HR, Hibbs JW, Crist, WL, Milk fever in dairy cows: effect of injected vitamin D3 and calcium and phosphorus intake on incidence. Int. J. Dairy Sci. 1977; 60: 431-436.

34. Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G. A body condition score chart for Holstein dairy cows. Int. J. Dairy Sci. 1989; 72: 68-78

35. Duffield TF, Leslie KE, Sandals D, Lissemore K, McBride BW, Lumsden JH, et.al. Effect of a monensin-controlled release capsule on cow health and reproductive performance. Int. J. Dairy Sci., 1999; 82(11): 2377–2384.



**How to cite this article**
Hassanabadi M, Mohri M, Sifi HA (2020). Effects of single injection of vitamin D3 on some immune and oxidative stress characteristics in transition dairy cows. Iran J Vet Sci Technol. 12(2):25-35
DOI: https://doi.org/10.22067/ijvst.2020.39239
URL: https://ijvst.um.ac.ir/article_39239.html